IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION

Examiner	:	Richard A. Schnizer
Group	:	1635
Applicants	:	Jeffrey Browning and Yves Chicheportiche
Application No.	:	09/245,198
Confirmation No.	:	4642
Filing Date	:	February 5, 1999
For	:	A TUMOR NECROSIS FACTOR RELATED LIGAND

Hon. Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

DECLARATION OF JEFFREY BROWNING, Ph.D.
UNDER 37 C.F.R. § 1.132

I, Jeffrey Browning, Ph.D., hereby declare and state as follows:

1. I am one of the named co-inventors of the above-identified patent application. I currently hold the

position of Distinguished Investigator at Biogen, Inc., now Biogen Idec MA Inc. ("Biogen"), the assignee of the above-identified patent application. I have been employed at Biogen, in Cambridge, Massachusetts, since 1984. During that time, I have served as Project Leader for the following projects: New TNF Members, BAFF and TWEAK; Development of Agonist LTβR mAb for Solid Tumors; Surface Lymphotoxin: Structure and Function and Studies on the Role of Exo-PLA2 in disease. I have also conducted investigations and evaluations of the anti-inflammatory properties of interferons and annexin-1, and have studied novel cytokines and the active domains of interleukin-2. From 1982 to 1984, I held the position of Senior Scientist at Angenics, Inc. in Cambridge, Massachusetts. My research there involved the development of anti-β-lactam monoclonal antibodies. From 1981 to 1982, I was a Postdoctoral Fellow in the laboratory of Dr. Louis Reichardt, Departments of Physiology and Biochemistry, University of California at San Francisco, working on applications of hybridoma technology to the study of intermediate filaments at neuromuscular junctions. From 1976 to 1981, I was a Postdoctoral Fellow in the laboratory

of Dr. Joachin Seeling, Department of Biophysical Chemistry, University of Basel, Switzerland. There, my work related to studies on phospholipids in membranes using solid state nuclear magnetic resonance methods.

2. I received a Ph.D. degree in Biochemistry from the University of Wisconsin in 1976. I received a B.S. degree cum laude in Biochemistry from Michigan State University in 1972. As illustrated by the bibliography in my curriculum vitae, which is attached as Exhibit A, I have been an active researcher in the fields of Biochemistry, Molecular Biology and Immunology. Further details of my education and research career are set forth in Exhibit A.

3. I have read and considered the above-identified application, as well as the July 22, 2003 Office Action ("the Office Action") in the application. I am informed and believe that the application claims an effective filing date of August 7, 1996.

4. I have read and considered claims 1-4, 6-8, 10, 28, 30, 31, and 39-47 of the above-identified application, which I am informed and believe were pending at the time of the Office Action. I understand that those claims stand rejected under 35 U.S.C. § 112, first

paragraph. Specifically, I understand that, in the Examiner's view, the claims are not supported by a description in the specification that enables a person of skill in the art to make and/or use the claimed invention without carrying out undue experimentation.

5. I make this declaration to demonstrate that a person of skill in the art, following the teachings of the above-identified application, would appreciate the biological function of Tumor Necrosis Factor Related Ligand ("TRELL"). Given that appreciation, a person of skill in the art would recognize the utility of and be able to use nucleic acids encoding TRELL polypeptides, as well as the TRELL polypeptides themselves, in the development of therapeutics, diagnostics or drug targets, as described in the application.

6. For the purpose of this declaration, "a person of skill in the art" as of August 7, 1996 would have a Ph.D. degree at the time and several years of relevant clinical and/or research experience involving Immunology.

7. In my opinion, a person of skill in the art would appreciate the biological function of nucleic acids encoding TRELL polypeptides and the TRELL polypeptides

themselves, as described in the above-identified application. In light of such appreciation, that person would be enabled to use those nucleic acids and the polypeptides they encode, in the development of therapeutics, diagnostics or drug targets, without resort to undue experimentation. My opinion is based on information and data presented in the application, as well as the confirmatory studies discussed below.

8. The above-identified application described TRELL (a then-new member of the tumor necrosis factor family of cytokines), the amino acid sequences of human and murine TRELL polypeptides, as well as the DNA sequences encoding those polypeptides. The application teaches that human TRELL is expressed in many tissues and organs of the immune system, such as the spleen, peripheral blood lymphocytes, lymph nodes, appendix, thymus, fetal liver and bone marrow (see, e.g., page 31, lines 4-11 and Figure 4). In addition, the application teaches that human TRELL is also expressed in organs of the secondary immune system, such as the ovary, prostate, small intestine, colon, heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas (see, e.g., page 31, lines 12-14 and Figure 4). Such expression

indicates an immunological function for TRELL (see, e.g., page 11, lines 33-34; page 13, lines 13-14 and 19-22 of the application). Based on such teaching, a person of skill in the art would appreciate a likely role of TRELL in the immune system, as well as immune-related disorders, such as cancer.

9. As discussed in the "Background of the Invention" section of the above-identified application, TNF family members function by binding to a receptor. A person of skill in the art would appreciate that, as is true for other TNF family members, a function of TRELL would involve its interaction of TRELL with a receptor. As taught at page 15, lines 16-19 of the above-identified application, TRELL polypeptides specifically interact with a receptor.

10. Pages 35-37 of the above-identified application teach that soluble human TRELL polypeptide can bind to a number of tumor cell lines, including K562 promyelocytic cells, THP-1 monocytic leukemic cells, 293 embryonic kidney cells, Cos kidney fibroblast cells and HT29 colon adenocarcinoma cells (see Table II on page 37 of the application). A person of skill in the art would appreciate that observed binding to denote: (1) the presence of TRELL

receptors on those tumor cells and (2) a potential role of TRELL in cancer pathology. That person would also appreciate that cancer therapy could be based on an agonistic or blocking interaction between TRELL and its receptor on tumor cells.

11. Further elucidating the role of TRELL in immune-related disorders, such as cancer, the application also describes the ability of human TRELL polypeptide to induce cytotoxicity in HT29 human adenocarcinoma cells (see page 8, lines 3-6; page 36, lines 1-10 and Table II of the application). A person of skill in the art would recognize, based on the binding properties of TRELL, coupled with its cytotoxic activity on tumor cells, that TRELL may be useful as an agonist in cancer therapy to promote tumor killing.

12. In the Office Action, the Examiner discounts the HT29-14 cytotoxicity assay, on the basis that the structure of the TRELL polypeptide used in the assay was not disclosed. That is not the case. The HT29-14 cytotoxicity assay is detailed at page 36, line 1 to page 37, line 28 of the above-identified application. As stated on page 36, lines 1-4, the HT29-14 cytotoxicity assay was carried out using human TRELL. In my view, it is apparent from the

application that the soluble human TRELL polypeptide produced in the Example on page 34, line 24 to page 35, line 9, was the TRELL polypeptide used in the HT29-14 cytotoxicity assay, as that Example immediately precedes the Example relating to the assay.

13. Citing Dermer, Biotechnology, 12: 320 (1994), the Examiner also discounts the relevance of the HT29-14 cell line to disease, based on the view that *in vitro* cell lines are a poor representation of malignancy, with characteristics profoundly different from human disease. In my opinion, that view would not be shared by a person of skill in the art.

14. For several reasons, a person of skill in the art would recognize the utility of the HT29-14 cell line for assessing a potential anti-tumor agent. The HT29-14 cell line represents a subclone (number 14) of conventional HT29 cells, that is routinely used to avoid culture drift. The properties of that cell line are identical to those observed in the parental HT29 tumor cell line maintained at the American Type Tissue Culture. The HT29 cell line resembles an early epithelial progenitor cell capable of differentiating into a number of specialized epithelial cell

types (Neutra et al.[1]). As such, it is similar to many primary human colorectal tumors. Based on additional work carried out at Biogen using gene chip analyses, gene profiling has shown that the HT29 cell line may resemble as much as 30% of the spectrum of primary colorectal tumors. In fact, during the period from 1976-1986, the National Cancer Institute adopted HT29 cells (called "CX-1") as its primary tumor screen for assessing efficacy of potential therapeutics for colon cancer (Plowman et al.[2]). Furthermore, development of other therapies has also relied substantially on the HT29 cell line, *e.g.*, the original work on the TNF family member lymphotoxin-α/β, which led to the development of an agonist anti-LT-β-R monoclonal antibody to mimic ligand binding (Browning et al.[3]). Thus, a person of skill in the art would appreciate the cell death inducing activity of human TRELL polypeptide on the HT29-14 tumor

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[1] Functional Epithelial Cells in Culture LIss: 363-398 (1989), a copy of which is provided at Exhibit B hereto.

[2] In Anticancer Drug Development Guide: Preclinical screening, clinical trials and approval. B. Teicher, ed. Humana Press Inc., Totown, 101-125 (1997), a copy of which is provided at Exhibit C hereto.

[3] J. Exp. Med. 183:867-878 (1996), a copy of which is provided at Exhibit D hereto.

cell line, as demonstrated in the above-identified application, to be indicative of the biological function of TRELL in cancer pathology.

15. On information and belief, I understand that additional work carried out at Biogen demonstrates the ability of soluble human TRELL polypeptide to induce cytotoxic activity in a number of tumor cell lines, other than in the HT29-14 cell line, either in the presence or absence of IFN-γ. Those additional cell lines include: WiDr colon adenocarcinoma cells, Geo colon carcinoma cells, MDA-MB-231 breast carcinoma cells, MX-1 breast carcinoma cells and the NCI-ADR breast carcinoma cells (data not shown). These additional cell lines have been routinely used in tumor screening panels. The colonic adenocarcinoma cell lines, HT29 and WiDr, were established originally from the same human patient, yet these tumor cell lines behave very differently in terms of their responsiveness to chemotherapy. The Geo colon tumor cell line has been included routinely in the National Cancer Institute's *in vitro* tumor screening panel for testing the efficacy of anti-cancer therapeutics for colon cancer. Similarly, the MX-1 breast carcinoma cell line has been used by the

National Cancer Institute in both its *in vivo* and later, *in vitro* tumor screens for assessing the efficacy of potential therapeutics for breast cancer. The MDA-MB-231 breast carcinoma cell line has also been included as part of the *in vitro* screening panel for therapeutics designed for the treatment of breast cancer. Based on the teachings in the above-identified application and the additional confirmatory studies discussed above, a person of skill in the art would appreciate that *in vitro* cell lines of different tissue origins are of use for assessing potential anti-tumor agents, such as TRELL.

16. In my opinion, for all of the reasons detailed above, it is simply not the case that *in vitro* cell lines, such as HT29-14, have little relevance to the *in vivo* disease state. Indeed, several anti-tumor therapeutics have been identified and evaluated based on activity in *in vitro* cell cultures. One such example is the highly successful anti-cancer drug Herceptin®, a humanized antibody approved for the treatment of HER2 positive metastatic breast cancer, which was developed following demonstration of strong efficacy *in vitro* against a limited number of tumor cell

lines (Shepard et al.[4]). Specifically, Shepard et al. discusses clinical application of anti-HER2 monoclonal antibodies for the treatment of breast cancer based on moderate to strong anti-proliferative activity against two *in vitro* tumor breast cell lines overexpressing the HER2 protoncogene ($p185^{HER2}$).

17. The biological activity of TRELL polypeptides taught in the above-identified application, and confirmed by the additional *in vitro* assays discussed above, is further confirmed by additional studies carried out at Biogen. On information and belief, I understand those studies to confirm the ability of antibodies directed to TRELL polypeptides to detect the expression of TRELL polypeptides in various diseases and conditions, as taught at page 16, line 17 to page 18, line 15 of the application.

18. Following the teachings of the application, additional studies at Biogen have led to a sensitive ELISA using anti-TRELL monoclonal antibodies to detect the level of TRELL polypeptide in the sera of patients suffering from diseases, such as lupus. The ELISA assay was based on a

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[4] J. Clin. Immunol. 11:117-127 (1991), a copy of which is provided at Exhibit E hereto.

hamster anti-human TRELL monoclonal antibody ("the BEB3 antibody") coupled with a biotinylated mouse anti-TRELL antibody ("the P5G9 antibody"). As illustrated in Figure 1 (Exhibit F hereto), the anti-TRELL antibodies detected significantly higher systemic levels of TRELL polypeptide in the sera of lupus patients (n=183), as compared with sera of control patients (n=42), $p<2.85E-06$. This sensitive ELISA provides not only a diagnostic tool for numerous diseases and conditions associated with TRELL but confirms the above-identified application's teaching of the role of TRELL in diseases related to the immune system. The ELISA is also useful in assays to detect TRELL polypeptide levels, for screening drug candidates which are either agonists or antagonists of the normal cellular function of TRELL or its receptor.

19. On information and belief, I understand that additional studies carried out at Biogen confirm a biological role of TRELL in the development of a number of other diseases *in vivo*, based on the ability of anti-TRELL antibodies to alleviate disease severity in conventional *in*

vivo models for arthritis (Bendele et al.[5]) and stroke (Martin-Vellalba et al.[6]) (data not shown).

20. The art has also confirmed that TRELL induces a range of proinflammatory mediators that may have anti-tumor activity. In general, induction of a pro-inflammatory program within a tumor environment can be beneficial, as it draws leukocytes into the tumor and provokes an immunological response. Generally, leukocytes, especially monocytes, do not penetrate effectively into the tumor environment. As is true for other TNF family receptors, activation of the TRELL receptor can trigger pro-inflammatory-like responses in various cells. In the case of TRELL, IL-8 release has been demonstrated (Chicheportiche et al.[7]), along with release of IP-10 and Mig, two chemokines that bind to the CXCR3 receptor (based on additional work carried out at Biogen). While the actual effects of releasing a spectrum of pro-inflammatory

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[5] Arthritis Rheum. 43:2648-2659 (2000), a copy of which is provided at Exhibit G hereto.

[6] Cell Death Differ. 8:679-686 (2001), a copy of which is provided at Exhibit H hereto.

[7] J. Biol. Chem. 272:32401-32410 (1997), a copy of which is provided at Exhibit I.

chemokines is difficult to predict, a person of skill in the art would appreciate that expression of some of these chemokines can lead to anti-tumor activity (Dias et al.[8]). For example, the CXCR3 binding chemokines have been reported to be both anti-tumor growth and anti-angiogenic (Tannenbaum et al.[9]). For these reasons, TRELL therapy may have clinical benefit by involving the immune system in addition to directing an anti-tumor effect.

21. The above-identified application describes on page 15, lines 16-19, that TRELL polypeptides specifically interact with an unidentified receptor. The application further teaches use of the disclosed peptides and methods to identify that receptor (page 31, line 17-page 32, line 12 of the application). A person of skill in the art, having TRELL polypeptide in hand, would be able to identify the receptor for TRELL polypeptide, as methods for identifying receptors for identified ligands were conventional at the time. For example, using soluble recombinant TRELL, Wiley

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[8] Cancer Invest. 19:732-738(2001), a copy of which is provided at Exhibit J hereto.

[9] J. Immunol. 161:927-932 (1998), a copy of which is provided at Exhibit K hereto.

et al.[10] identified the fibroblast growth factor-inducible 14 (Fn14) as a TRELL receptor which was responsible for TWEAK-induced proliferation of endothelial cells and angiogenesis.

22. In my opinion, the above-identified application, read in light of the state of the art at the time, teaches a person of skill in the art the biological function of TRELL. In view of such teaching, the application provides a nexus between TRELL and its biological function in the development and progression of a number of immune-associated diseases and enables a person of skill in the art to use nucleic acids encoding TRELL polypeptides, and the TRELL polypeptides which they encode, in therapies and diagnostics directed to such diseases. Further, the application enables such uses without resort to undue experimentation.

23. I declare further that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the

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[10] Immunity 15:837-846 (2001), a copy of which is provided at Exhibit L hereto.

knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

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Jeffrey Browning, Ph.D.

Signed at Cambridge, Massachusetts,
this _____ day of _____, 2004